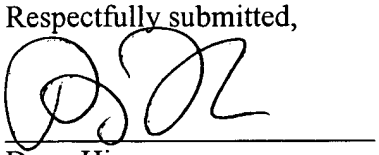
Therefore, no new matter has been added. Entry and consideration of this Amendment is respectfully requested.

Respectfully submitted,



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APPENDIX

VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE SPECIFICATION:

The specification is changed as follows:

Page 21-22 Third full paragraph:

The hybridization can be carried out under the aforementioned stringent condition. For example, DNAs from a genomic DNA library or a cDNA library obtained from a microorganism capable of producing an enzyme of the present invention is fixed on nylon membranes, and the thus prepared nylon membranes are subjected to blocking at 65°C in a pre-hybridization solution containing 6 x SSC, 0.5% SDS, 5 x Denhart's and 100 μg/ml of salmon sperm DNA. Thereafter, each probe labeled with ^{32}P or digoxigenin is added thereto, followed by incubation overnight at 68°C. The thus treated nylon membranes are washed in 6 x SSC containing 0.1% SDS at room temperature for 10 minutes, in 6 x SSC containing 0.1% SDS 45°C for 30 minutes and then subsequently subjecting the thus washed membranes to an auto-radiography or detection of digoxigenin to detect a DNA fragment which hybridizes with the probe in a specific fashion. Also, genes which show various degree of homology can be obtained by changing certain conditions such as washing or lowering the hybridization temperature (e.g., 45°C).

Page 22 2nd full paragraph:

In order to determine whether the thus obtained gene encodes a polypeptide having the enzyme activity of interest, the thus determined nucleotide sequence is compared with the nucleotide sequence coding for the enzyme of the present invention or with its amino acid sequence, and the identity is estimated based on the gene structure and homology. Alternatively,

it is possible to determine whether the gene encodes a polypeptide which has the enzyme activity of interest by producing a polypeptide ~~of~~ encoded by the gene and measuring its enzyme activity.

Page 32, Second full paragraph:

Example 2

In accordance with Example 1, *Aspergillus fumigatus* IAM 2046 was pre-cultured in the same manner, and the resulting culture broth was inoculated into a main culture medium (2% soybean flour, 0.3% sodium chloride, 0.1% KH_2PO_4, 0.05% $MgSO_4 \cdot 7H_2O$, 3% soluble starch, 0.5% gentose #80 (mfd. by Nihon Shokuhin Kako), pH 5.6) and cultured for 4 days to obtain a crude enzyme solution. As the result, the

Page 33, Table 3:

Table 3

Inducers	Inducing ability (%)
Not added	100
Isomaltose	145
Maltotriose	171
Maltose	136
Gentose #80	235
Gentiobiose	211
Gentio-oligosaccharide	180
Sucrose	116
Trehalose	113
Glucose	164
Galactose	125
Fructose	143
Rhamnose	129
Tulbose	116
Maltitol	142
Arabitol	112
Galactitol	142
Glucosamine hydrochloride	157

Page 48, First full paragraph:

Its optimum temperature was measured in the following manner. A 400 μl portion of 2 mM pNP-primeveroside solution prepared using 20 mM ~~secondary~~ disodium citric acid-HCl buffer (pH 2.5) was mixed with 90 μl of the enzyme solution to carry out the reaction at 30 to 65°C for 20 minutes. The reaction was stopped by adding 500 μl of 0.5 M sodium carbonate solution, and the activity was determined by measuring the absorbance at 420 nm. It was found that sufficient activity is maintained, because diglycosidase derived from *Aspergillus fumigatus* has 80% of the activity even at 60°C, in comparison with the plant-derived enzymes having similar activity.

Page 48, Second full paragraph:

Its pH stability was measured in the following manner. The purified enzyme preparation was diluted 100 times with each of ~~secondary~~ disodium citrate buffer of from pH 2 to 5, phosphate buffer of from pH 6 to 8 or glycine NaCl-NaOH buffer of from pH 7 to 10 and treated at 37°C for 1 hour, and then a 90 μl portion thereof was mixed with 400 μl of 2 mM pNP-primeveroside solution (pH 2.5) which had been incubated at 37°C for 5 minutes, and the reaction was carried out at 37°C for 20 minutes. The reaction was stopped by adding 500 μl of 0.5 M sodium carbonate

Page 63, First full paragraph:

In order to verify whether or not the cloned gene is the primeverosidase gene, expression of the thus obtained DNA was confirmed. Using an *Aspergillus oryzae* Taka-amylase gene-containing plasmid pTG-Taa (Kato M, Aoyama A, Naruse F, Kobayashi T and Tsukagoshi N

(1997), An *Aspergillus nidulans* nuclear protein, An CP, involved in enhancement of Taka-amylase A gene expression binds to the CCAAT-containing *taaG2*, *amdS* and *gatA* promoters., *Mol. Gen. Genet.*, 254: 119 - 126) as the template, a fragment was obtained by amplifying it by PCR using

a primer TAA5'

SEQ ID NO: ~~10~~ 11

sense primer:

5'-GGGCCTGCAGGAATTCATGGTGTT-3'

and a primer TP3'

SEQ ID NO: ~~11~~ 12

antisense primer:

5'-CGAGCCGGGGTTTCCGTCCGCAGGCGTTGC-3'.

<PCR reaction solution>

template DNA solution (50 μg/ml)	1 μl
50 μM sense primer	1 μl
50 μM antisense primer	1 μl

Page 64, First full paragraph:

Also, a fragment was obtained by amplifying it by PCR using the DNA-containing plasmid pAFPri as the template and using a primer dPC5'

SEQ ID NO: ~~12~~ 13

sense primer:

5'-GCAACGCCTGCGGACGGAAACCCCGGCTCG-3'

and a primer PC3'

SEQ ID NO: ~~13~~ 14

antisense primer:

5'-GCGCAAGCTTGGAAGCTGCACGACATGTAA-3'.

Page 65, First full paragraph:

Regarding the terminator region, a fragment was obtained by amplifying it by PCR using pTG-Taa as the template and using a primer TAAH

SEQ ID NO: ~~14~~ 15

sense primer:

5'-GCGCAAGCTTTGAAGGGTGGAGAGT-3'

and a primer TAA3'

SEQ ID NO: ~~15~~ 16

antisense primer:

5'-GCGCCCTGCAGGTCTAGAATTCCTAGTGGTT-3'.

Page 66-67, Second paragraph:

An orotidine-5'-phosphate decarboxylase ~~(pyrG)~~ (PyrG) p r oducing strain *Aspergillus nidulans* G191 (Kato M (1997), *Mol. Gen. Genet.*, 254: 119 - 126) was inoculated into a complete medium (2% malt extract, 0.1% peptone, 2% glucose, 0.1% uridine, 2 μg/ml p-aminobenzoic acid, pH 6.5) and cultured at 30°C for 18 hours on a shaker. The cells were collected by filtration, suspended in a protoplast solution (0.8 M NaCl, 10 mM NaH_2PO_4, 20 mM $CaCl_2$, 3.75 mg/ml Novozyme 234) and then treated at 30°C for 1 hour on a shaker. The resulting protoplasts were recovered by filtration and centrifuged at 1,500 rpm for 5 minutes to obtain the protoplasts as the precipitate. This precipitate was suspended in 0.8 M NaCl solution

and centrifuged at 1,500 rpm for 5 minutes to collect the precipitate. This was again suspended in 0.8 M NaCl/50 mM $CaCl_2$ solution and centrifuged at 1,500 rpm for 5 minutes to collect the precipitate. A protoplast solution was obtained by suspending this in an appropriate amount of 0.8 M NaCl/50 mM $CaCl_2$ solution. Next, 50 μl of this protoplast solution was mixed with 20 μg of a DNA solution and 12.5 μl of a PEG solution (25% PEG 6000/50 mM $CaCl_2$/10 mM Tris-HCl (pH 7.5)) and then allowed to stand for 20 minutes on ice. Next, 0.5 ml of PEG solution was added and then the mixture was allowed to stand for 5 minutes on ice. Next, 1 ml of 0.8 M NaCl/50 mM $CaCl_2$ solution was added and mixed. A 0.5 ml portion of this mixed solution was mixed with 15 ml of 2% agar-containing regeneration medium (0.6% $NaNO_3$, 11 mM KH_2PO_4, 7 mM KCl, 1.2 M sorbitol, 0.05% $MgSO_4 \cdot 7H_2O$, 1% glucose, 2 μg/ml p-aminobenzoic acid, pH 6.5) which had been incubated at 50°C in advance, solidified in Petri dishes and then cultured at 37°C for 3 days.

Page 76, First full paragraph:

Example 16

Hydrolysis of other disaccharide glycosides

Using the diglycosidase preparations derived from various microorganisms shown in Example 5, their ability to hydrolyze various disaccharide glycosides was examined using TLC. As a result, it was revealed that the diglycosidase acts upon not only the primeveroside glycosides but also various other disaccharide glycosides analogous to the primeveroside glycosides, including rutinose glycosides such as naringin and rutin, gentiobiose glycosides, arabinofuranosyl glycosides and ~~aviofuranosyl~~ apiofuranosyl glycosides, and thereby releases disaccharides and produces respective free aglycons.

Page 79, Second full paragraph:

The purified enzyme of Example 6 was diluted with deionized water to adjust the activity to 1.0 AU/ml, and acetonitrile (200 ~~ml~~ l) containing 2.5% phenethyl alcohol, 20 mM acetate buffer (250 μl) containing 10% primeverose and the enzyme solution (50 μl) were mixed to carry out the reaction at 55°C. The reaction was completed after 6 hours and the reaction solution was mixed with 500 μl of diethyl ether, stirred and then centrifuged to remove free aglycon which was transferred into the ether layer. By applying the water layer to a Diaion HP-20 column and passing purified water through the column, free primeverose was removed. The disaccharide glycoside adsorbed to the resin was eluted with methanol and concentrated to dryness. This was dissolved in 100 μl of deionized water, and a 20 μl portion thereof was spotted on a TLC plate to detect the reaction product (the developing solvent was ethyl acetate:acetic acid:deionized water = 3:1:1, acetic